Phage phi 29 protein p1 promotes replication by associating with the FtsZ ring of the divisome in Bacillus subtilis

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Supporting Information

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SI Materials and Methods

Plasmids Construction. Plasmids used in this work are shown in Table S7. To generate plasmid pSG5472/YFP-p1, gene *1* of *Bacillus subtilis* phage ϕ29 was amplified by PCR with primers YFPP1-XhoI (5′-GAGATGCTCGAGATGGGTAAAATCTTCGATCAAG-3′) and YFPP1-EcoRI (5′-GAGTCAGAATTCTCATTTTGCACC-ATGTGGAGC-3′). The resultant product was digested with restriction enzymes XhoI and EcoRI and subsequently ligated to plasmid pSG5472 previously digested with the same enzymes. *Escherichia coli* cells were transformed with this ligation mixture, and ampicillin-resistant transformants were selected. *B. subtilis* 168 cells were transformed with plasmid pSG5472/YFP-p1, and spectinomycin-resistant transformants were tested for their ability to degrade starch to select for double cross-over transformants. Plasmid pDP150/FtsZ-CFP was constructed as follows. The DNA fragment corresponding to *ftsZ-cfp*, flanked by NheI and SphI restriction sites, was amplified by PCR from genomic DNA of *B. subtilis* strain 1057 (1) (primers 5′-TCGATAGCTAGCTCTA-GAAAGGAGATTCCTAGGATG-3′ and 5′-CGTGATGCATG-CTTACTTGTACAGCTCGTCCAT-3′), digested with restriction enzymes NheI and SphI, and cloned into equivalent sites of the thrC-integrating vector pDP150, which contains the isopropyl-β-D-1-thiogalactopyranoside (IPTG) inducible $P_{hyper\text{-}spank}$ promoter. To generate plasmid pDP150/p1, vector pDP150 was digested with NheI and SphI and ligated with the NheI- and SphI-digested DNA fragment containing gene *1* of *B. subtilis* phage ϕ29. This fragment was generated by PCR using primers p1-UNheI (5′-AACGTGC-TAGCAAAAGGAGATGTTTGTAATGGGTAAAATC-3′) and p1-LSphI (5′-AGTCAGCATGCTCATTTTGCACCATGTG-GAGC-3′). Plasmid pT7-3/p1ΔC11 was generated by using the QuikChange Site-Directed Mutagenesis Kit (Stratagene) with primers p1ΔC11-5′ (5′-CGCCACATGGGTTATAAAGT-AATAGAAGGATGACGATGACAAG) and p1ΔC11-3′ (5′-CTTGTCATCGTCATCCTTCTATTACTTTATAACCCATG-TGGCG-3′) to introduce a stop codon 11 triplets before the natural stop codon of ϕ29 gene *1*.

Purification of Proteins p1ΔC11 and Filamenting Temperature-Sensitive Mutant Z Protein (FtsZ). A frozen pellet of p1ΔC11-containing *E. coli* BL21-p1ΔC11 cells (50 g) was ground with 100 g of alumina for 25 min. The resultant lysate was diluted by adding four volumes of buffer P [50 mM Tris·HCl, pH 7.5, 1 mM EDTA, 7 mM β-mercaptoethanol, and 5% (vol/vol) glycerol] containing 0.1 M NaCl. This mixture was centrifuged for 5 min at 2,500 × *g* to remove alumina and intact cells. A second centrifugation at 15,000 × *g* for 15 min was performed, and the supernatant was diluted to 120 A_{260} units/mL with buffer P containing 0.1 M NaCl. Next, 10% (wt/vol) polyethyleneimine was added to a final concentration of 0.3% (wt/vol). The solution was centrifuged at 15,000 × *g*, and the supernatant was precipitated with ammonium sulfate to 50% saturation. After centrifugation at 16,000 × *g* for 10 min, the pellet was resuspended in buffer Q [50 mM Tris·HCl, pH 7.5, 1 mM EDTA, 7 mM β-mercaptoethanol, 5% (vol/vol) glycerol, and 33% (wt/vol) ammonium sulfate] and then centrifuged again for 10 min at 16,000 × *g*. The pellet was resuspended in buffer P to reach a 25 mM ammonium sulfate concentration and then loaded onto a Mono Q Sepharose column previously equilibrated with buffer P containing 25 mM NaCl. The column was washed twice with buffer P containing 20 and 25 mM NaCl, respectively, and was eluted with buffer P containing 0.1 M NaCl. The eluate was precipitated with ammonium sulfate to 65% saturation. Protein FtsZ was purified as described previously (2).

Fluorescence Microscopy Experiments. To determine the intracellular distribution of YFP-p1 expressed in *B. subtilis* DBP-001, overnight cultures [in Luria-Bertani (LB) medium containing 100 μg/mL spectinomycin, 5 μg/mL kanamycin, and 5 mM $MgSO_4$] were diluted 1:100 in the same fresh medium and grown at 37 °C to an OD_{600} of 0.45. Then, xylose was added to a final concentration of 0.5% (wt/vol), and half of the culture was infected with phage ϕ29 at a multiplicity of infection (MOI) of 5. Thirty minutes after infection and induction, cultures were collected, and cells were immobilized onto microscope slides covered with a thin film of 1% (wt/vol) agarose. *N*-(3-triethylammoniumpropyl)-4-(6-(4-(diethylamino) phenyl)hexatrienyl)pyridinium dibromide (FM 4-64; Invitrogen) was used to stain the bacterial membrane. The same procedure was used to visualize the localization of YFP-p1 in the presence and absence of protein FtsZ or PBP2B. LB medium contained 100 μg/mL spectinomycin and 1 μg/mL phleomycin for *B. subtilis* strain DBP-003 and 100 μg/mL spectinomycin, 5 μg/mL neomycin, and 5 μg/mL chloramphenicol for *B. subtilis* strain DBP-004; 0.5 mM IPTG and 0.5% (wt/vol) xylose was added to analyze YFP-p1 localization under a WT background.

To determine colocalization of YFP-p1 and FtsZ-CFP, an overnight culture of strain DBP-002 grown in LB medium with 100 μg/mL spectinomycin and 1 μg/mL erythromycin was diluted 1:100 in the same fresh medium and grown at 37 °C to an OD_{600} of 0.45. At this OD, xylose and IPTG were added at final concentrations of 0.5% (wt/vol) and 1 mM, respectively. After 30 min, cells were collected and immobilized on agarose as mentioned above.

For immunofluorescence microscopy, overnight cultures of *B. subtilis* 110NA were diluted in LB medium containing 5 mM $MgSO_4$ and grown to exponential phase at 37 °C. At an OD_{600} of 0.5–0.6, the culture was infected with WT phage ϕ29 at a MOI of 5. Samples were fixed after 20 and 30 min and processed essentially as described previously (3). Polyclonal antibodies were centrifuged for 10 min at 14,000 × *g* at 4 °C before use to precipitate possible antibody aggregates. Affinity-purified rabbit polyclonal antibodies against p1 were used at a dilution of 1:500, and incubations were carried out for 1 h at room temperature. Polyclonal FITC-conjugated anti-rabbit antibodies were used at a 1:1,000 dilution and incubated at 4 °C overnight. These and all subsequent steps were performed with minimal exposure of the samples to light. All samples were mounted for epifluorescence microscopy in multispot microscope slides (C.A. Hendley) supplemented with 0.2 μg/mL DAPI.

Pull-Down Assay. *B. subtilis* strain 168His and strain 168Δ*spo0A* were grown overnight at 30 °C in LB medium supplemented with 5 μg/mL kanamycin and 0.5 mM IPTG (also with 0.5 μg/mL erythromycin in the case of 168His). Then, overnight cultures were diluted 1:100 in LB medium supplemented with 5 mM $MgSO_4$ and 0.5 mM IPTG and grown at 37 °C to an OD_{600} of 0.45. At this OD, half of each culture was infected with the mutant phage ϕ29 *sus14*(1242), and the cultures were grown during 30 min at 37 °C. A quarter of each culture was kept at 4 °C, and the rest was incubated with formaldehyde [1% (wt/vol) final concentration] for 30 min at 37 °C. Then, all cells were centrifuged at 11,900 × *g* for 5 min, resuspended in 12 mL TBS buffer (50 mM Tris·HCl, pH 7.5, 150 mM NaCl) and centrifuged again at 11,900 × *g* for 3 min. All pellets were stored at −80 °C. To pull-down, a portion of the cells were resuspended in 3 mL UT-buffer [100 mM Hepes, 500 mM NaCl, 50 mM imidazole,

8 M urea, 1% (wt/vol) Triton X-100, 1 mM DL-DTT (DTT), and 1 mM PMSF] and sonicated at 4 °C. After centrifugation of the samples at 7,750 × *g* for 10 min, each supernatant was incubated overnight with 200 μL of MagneHis suspension (Promega) at room temperature. Then, the resultant suspension was centrifuged at 2,800 × *g* for 1 min at room temperature, and the supernatant was discarded. MagneHis particles were washed 15 times with UT-buffer, and elution was performed with 200 μL of elution buffer [0.1 M Tris·HCl, pH 7.5, 0.5 M imidazole, 1% (wt/vol) SDS, and 10 mM DTT]. To remove nonspecifically bound or un–cross-linked proteins, the eluate was passed through a Microcon-100 column (Millipore), thus removing proteins with molecular mass lower than 100 kDa. Protein complexes that were retained on the membrane were washed three times with M-wash buffer [0.1 M Tris·HCl, pH 7.5, 1% (wt/vol) SDS, 10 mM DTT] and recovered by addition of 50 μL of the same buffer. Samples were resuspended in R buffer [0.5 M Tris·HCl, pH 6.8, 2% (wt/vol) SDS, 4% (vol/vol) β-mercaptoethanol, 13% (vol/vol) glycerol], heated at 90 °C for 1 h to revert the cross-linking, and subjected to tricine-SDS/PAGE (4) and Western blotting with anti-p1 antibodies.

In Vitro Cross-Linking. Proteins FtsZ and p1ΔC11 were incubated together or alone at a final concentration of 5 μM in HM buffer (50 mM Hepes, pH 7.5, 5 mM $MgSO_4$) for 30 min at 4 °C. GTP was added to a final concentration of 1 mM before incubation during 20 min at 37 °C. Then, the mixture was divided in two portions. DSS (final concentration of 1.25 mg/mL) was added to one half. Next, samples were incubated at 4 °C for 20 min with occasional shaking. Glycine was added to a final concentration of 250 mM to stop the cross-linking reaction and the mixture was incubated at 4 °C for 5 min. One volume of 20% (vol/vol) trichloroacetic acid (TCA) was added to the samples, and they were kept for 20 min at 4 °C before centrifugation at 22,000 × *g* for 10 min. TCA pellets were resuspended in a buffer composed of 37 mM Tris·HCl, pH 6.8, 2% (wt/vol) SDS, 4% (vol/vol) β-mercaptoethanol, and 13% (vol/vol) glycerol. Next, 1 M Tris·HCl, pH 7.5, was added to the samples to neutralize the acid pH. The samples were loaded onto a SDS/PAGE 10–20% gradient and analyzed by Western blot with antibodies against p1 and FtsZ.

***Bacillus subtilis* Cellular Length Measurements.** To determine the length of gene *1*–expressing *B. subtilis* cells, strains DBP-005 and DM-024 were grown overnight at 37 °C in LB medium supplemented with 1 μg/mL erythromycin (final concentration). Then, cells were diluted 1:400 in the same fresh medium and grown at 37 °C. When the culture reached an OD_{600} of 0.1, 1 mM IPTG was added. As an internal control, one half of the DBP-005 culture was not induced. After 45 min (approximately two generations), cells were stained with FM 4-64 and photographed with a laser scanning multiphoton microscope (Zeiss LSM710 coupled to an inverted AxioObserver and Zeiss LSM510 coupled to a vertical AxioImager M1). The same method was used to measure the length of *B. subtilis* strain 168Δ*spo0A* infected with phage ϕ29 *sus14*(1242) at a MOI of 1, but samples were collected at 50 min postinfection. As control, *B. subtilis* strain 168Δ*spo0A* was not infected.

Quantification of Viral DNA Synthesis. Primer sets R-25 (5′-AAAGTAGGGTACAGCGACAACATAC-3′) and R-OUT-SUPER (5′-AAATAGATTTTCTTTCTTGGCTAC-3′) were used to amplify a 297-bp fragment corresponding to the right end of the ϕ29 genome, and the primer sets ftsZ-R (5′-CAGTGATTGCAACCGGCTTTATCG-3′) and ftsZ-L (5′-TTTTGGCTCACGCTTCGGAACA-3′) were used to amplify a fragment of the *ftsZ* gene of the genomic *B. subtilis* DNA. The data obtained were interpolated to standard curves constructed with known amounts of phage and genomic DNA essentially as described previously (5). As control of the number of ϕ29-infected cells per milliliter of culture, a sample of the infected cells was centrifuged, washed twice to eliminate nonadsorbed phages, and diluted 1/1,000. One hundred microliters of the diluted culture was mixed with 500 μL of exponentially growing *B. subtilis* 110NA and top agar (0.75% agar) and plated on LB plates. To obtain the mean of plaque forming units per milliliter of culture, a minimum of eight platings was made for each strain in each experiment.

1. Feucht A, Lewis PJ (2001) Improved plasmid vectors for the production of multiple fluorescent protein fusions in *Bacillus subtilis*. *Gene* 264(2):289–297.
2. Scheffers DJ (2008) The effect of MinC on FtsZ polymerization is pH dependent and can be counteracted by ZapA. *FEBS Lett* 582(17):2601–2608.
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5. González-Huici V, Salas M, Hermoso JM (2004) The push-pull mechanism of bacteriophage Ø29 DNA injection. *Mol Microbiol* 52(2):529–540.

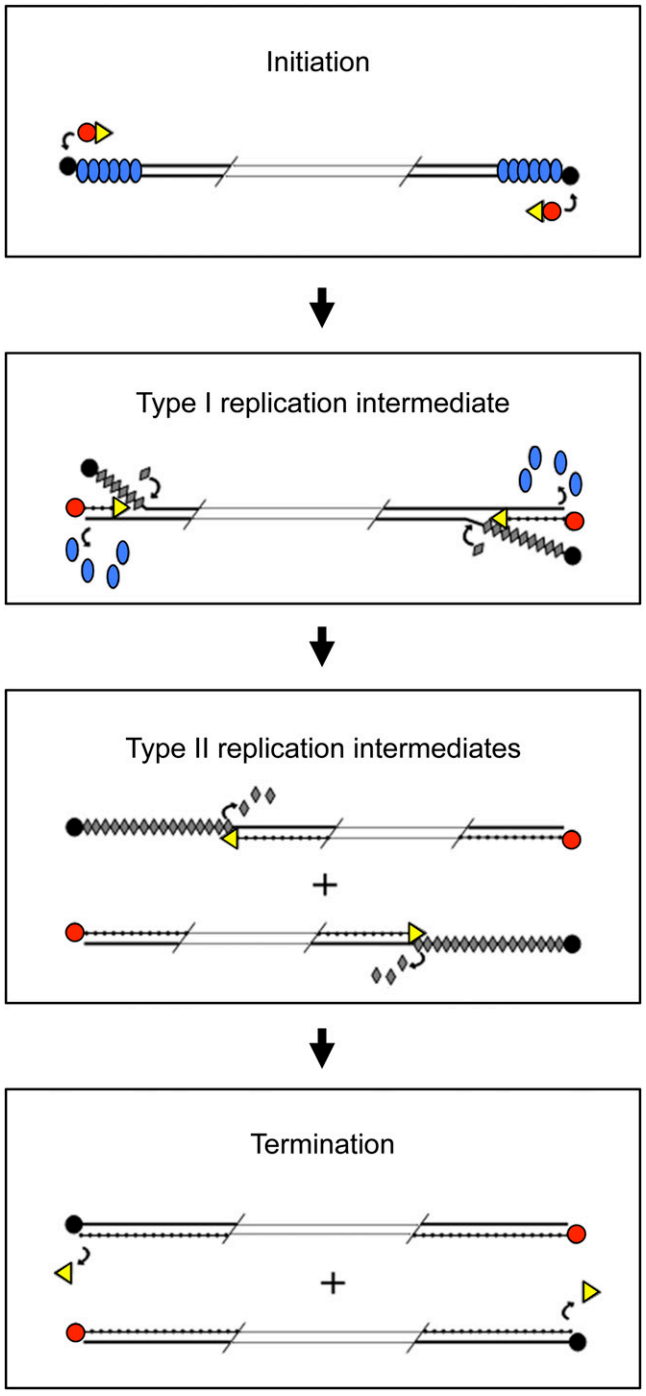

Fig. S1. Mechanism of in vitro ϕ29 DNA replication. Replication starts by recognition of the phage p6-nucleoprotein complexed origins of replication by a terminal protein (TP)/DNA polymerase heterodimer. Then, the DNA polymerase catalyzes the addition of the first dAMP to the TP present in the heterodimer. After a transition step, the DNA polymerase dissociates and continues processive elongation that is coupled to strand displacement. The ϕ29-encoded SSB protein binds to the displaced ssDNA and is further removed by the DNA polymerase. Continuous elongation of the DNA polymerase from both DNA ends generates replication intermediates that finally converge in the complete duplication of the parental strands (1, 2). Red circles, TP; yellow triangles, DNA polymerase; blue ovals, replication initiator protein p6; gray diamonds, SSB protein; de novo synthesized DNA is shown as beads on a string.

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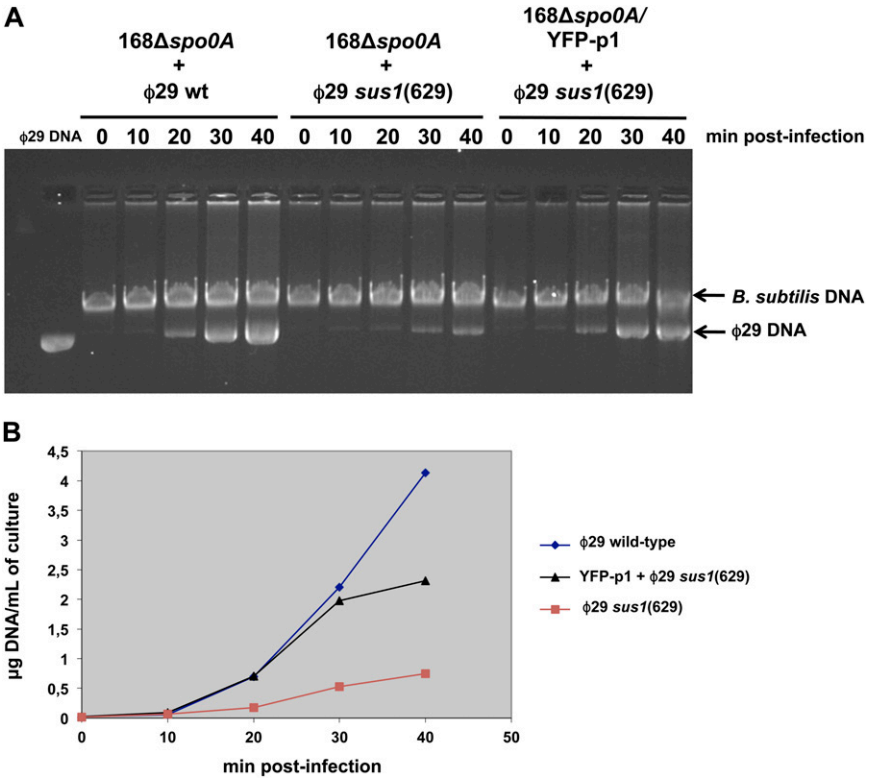

Fig. S2. YFP-p1 is functional in *B. subtilis* cells. (*A* and *B*) Complementation experiments using *sus1*(629) mutant phage infecting strain DBP-001 (168Δ*spo0A*/YFP-p1) containing a *yfp-p1* cassette under a xylose-dependent promoter. As an internal control, *B. subtilis* 168Δ*spo0A* strain was infected with either ϕ29 WT or *sus1* (629) mutant phage and the production of viral DNA was analyzed at the indicated times postinfection. (*A*) Agarose gel electrophoresis analysis illustrating the amount of viral DNA accumulated in the strains indicated above at different times postinfection. Arrows indicate positioning of the ϕ29 genome and *B. subtilis* chromosomal DNA. (*B*) The amount of intracellular accumulated viral DNA was quantified by real-time PCR, essentially as previously described (1).

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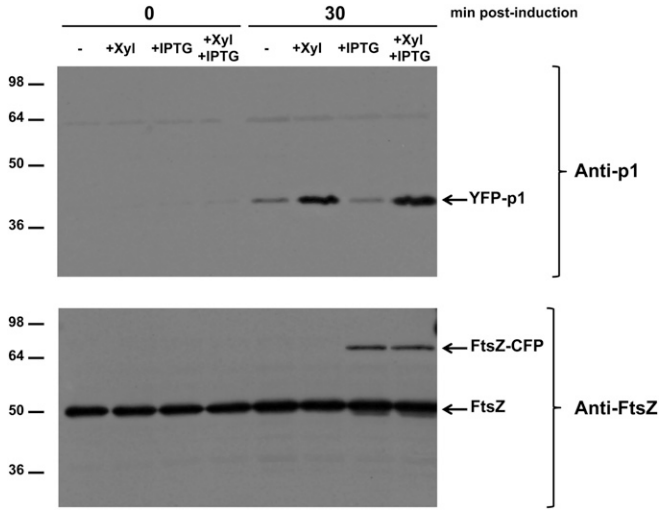

Fig. S3. Western blot analysis showing the induction of YFP-p1 and FtsZ-CFP at 30 min after addition of xylose (+Xyl) and IPTG (+IPTG). *B. subtilis* strain DBP-002 was grown in LB medium to exponential phase (OD_{600} of 0.45) at 37 °C. Then, xylose, IPTG or xylose plus IPTG were added to induce the synthesis of YFP-p1, FtsZ-CFP or YFP-p1 and FtsZ-CFP, respectively. At 0 and 30 min postinduction, whole cell lysates were analyzed using anti-p1 or anti-FtsZ antibodies.

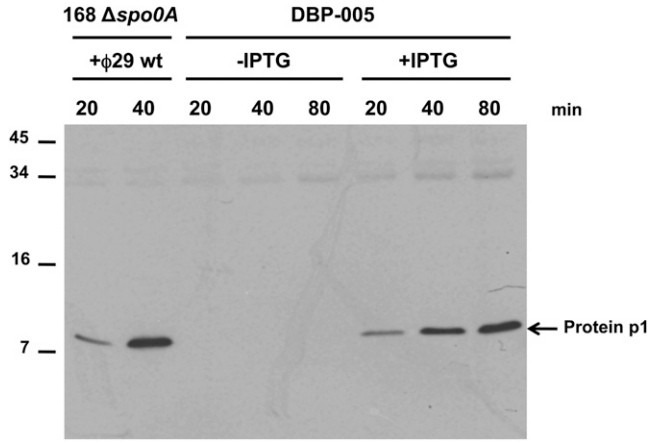

Fig. S4. Ectopic expression of protein p1 compared with the production in a phage ϕ29 infection cycle. *B. subtilis* 168Δ*spo0A* and DBP-005 (168Δ*spo0A* containing a ϕ29 gene *1* cassette under an IPTG-dependent promoter) strains were grown at 37 °C in LB medium to an OD_{600} of 0.1. 168Δ*spo0A* cells were then infected with WT ϕ29 at a MOI of 1. This culture and that of DBP-005 cells were simultaneously supplemented with 1 mM IPTG. At the indicated times (minutes), cell samples were harvested, processed, and subjected to SDS/PAGE and Western blotting with anti-p1 antibodies.

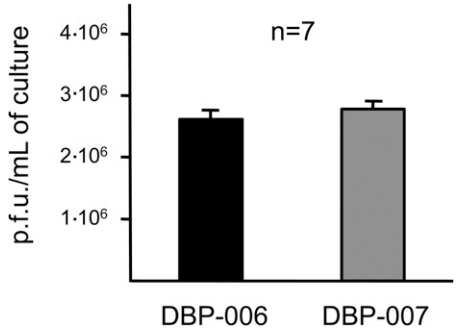

Fig. S5. WT and *ezrA*(*R510D*) mutant strains are infected similarly by phage ϕ29. Quantification of the number of ϕ29-infected cells per milliliter of culture was performed immediately after infection. A sample of the infected cells was centrifuged, washed twice to eliminate nonadsorbed phages, and plated on LB plates mixed as indicated in *SI Materials and Methods*. Phage plaques were counted and the number of plaque forming units (p.f.u.) per milliliter of culture in each strain was determined by obtaining the mean of a minimum of 8 platings.

Table S1. Cellular length in ϕ29 protein p1-expressing cells

Statistical parameters	−p1	+p1	+CFP
Number of values	242	121	133
25% percentile	3.10	4.52	3.24
Median	3.68	5.04	3.72
75% percentile	4.37	6.60	4.36
Mean	3.78	5.55	3.82
SD	0.87	1.47	0.76
SE	0.06	0.13	0.07

B. subtilis cells containing IPTG-inducible protein p1 (strain DBP-005) were grown to exponential phase and supplemented with 1 mM IPTG (final concentration). After 45 min (approximately two generations), cells were stained with FM 4-64 and photographed, and the cell length was measured with Fiji software (ImageJ) (*Materials and Methods*). As internal controls, half of the culture was not induced and *B. subtilis* cells containing IPTG-inducible CFP (cyan fluorescent protein) were induced with 1 mM IPTG (final concentration).

Table S2. Cellular length in *sus14*-infected cells

Statistical parameters	Noninfected	+ sus*14*(1242)
Number of values	175	175
25% percentile	2.96	3.68
Median	3.72	5.05
75% percentile	4.80	6.51
Mean	3.90	5.49
SD	1.16	2.54
SE	0.09	0.19

B. subtilis strain 168Δ*spo0A* cells were grown to exponential phase and infected with ϕ29 *sus14*(1242). After 50 min, cells were stained with FM 4-64 and photographed, and the cell length was measured with Fiji software (ImageJ) (*Materials and Methods*). As control, half of the culture was not infected.

Table S3. Number of ϕ29-infected cells in WT and *ezrA*(*R510D*) mutant strains

	Number of ϕ29-infected cells/mL of culture	
Experiment number	DBP-006 (WT)	DBP-007 [*ezrA*(*R510D*)]
1	3.4×10^6	3.3×10^6
2	1.2×10^6	3.2×10^6
3	2.9×10^6	2.8×10^6
4	3.5×10^6	2.4×10^6
5	2.4×10^6	2.5×10^6
6	2.7×10^6	3.0×10^6
7	2.1×10^6	2.6×10^6
Mean	2.6×10^6	2.8×10^6
SEM	3.0×10^5	1.3×10^5

Quantification of the number of ϕ29-infected cells per milliliter of culture was performed immediately after infection. A sample of the infected cells was centrifuged, washed twice to eliminate nonadsorbed phages, and plated on LB plates mixed with liquid top agar. Phage plaques were counted and the number of plaque forming units (p.f.u.) per milliliter of culture in each strain was determined by obtaining the mean of a minimum of eight platings.

Table S4. ϕ29 DNA accumulation in WT and *ezrA*(*R510D*) mutant strains

	Minutes postinfection					
	0		40		50	
Experiment	DBP-006 (WT)	DBP-007 [ezrA(R510D)]	DBP-006 (WT)	DBP-007 [ezrA(R510D)]	DBP-006 (WT)	DBP-007 [ezrA(R510D)]
1	0.0048	0.0034	1.8055	2.5041	2.2607	3.2745
2	0.0034	0.0033	1.6160	3.1810	2.2742	3.3657
3	0.0026	0.0024	1.5443	1.5151	2.0998	2.4785
4	0.0027	0.0024	1.2755	1.5470	1.7355	2.4867
5	0.0025	0.0020	1.0348	1.3176	1.7950	2.1621
6	0.0025	0.0018	0.9315	1.2382	1.6042	1.8636
7	0.0024	0.0021	0.9137	1.0185	1.5642	1.8416
Mean	0.0030	0.0025	1.3030	1.7602	1.9048	2.4961
				35.1%		31.0%

The amount of intracellular ϕ29 DNA at 0, 40, and 50 min postinfection of DBP-006 (WT) and DBP-007 [*ezrA*(*R510D*) mutant] strains was quantified by real-time PCR loading triplicates of each sample. Data (μg/mL of viral DNA) were obtained from seven experiments and normalized according to the same amount of genomic DNA of the infected strains. The difference in viral DNA accumulation between the mutant and WT strains at 40 and 50 min postinfection is given as percentage.

Table S5. Strains used

Strain	Relevant genotype*	Construction, source or reference†
E. coli		
DH5α	φ80d*lac*ZΔM15, *rec*A1, *end*A1, *gyr*AB, *thi*-1, *hsdR*17(r_K-, m_K+), *supE*44, *rel*A1, *deo*R, Δ(*lacZYA-arg*F) U169, *pho*A	Laboratory stock
XL1-Blue	*rec*A1, *end*A1, *gyr*A96, *thi*-1, *hsd*R17(r_K-, m_K+), *sup*E44, *rel*A1, *lac*, [F′, *proAB*, *lac*I^qZΔM15::Tn10(tet^r)]	Laboratory stock
BL21(DE3)	F–, *omp*T, *hsd*S_B(r_B-, m_B-), *dcm*, *gal*, λ(DE3)	Laboratory stock
BL21-p1ΔC11	BL21(DE3) containing plasmid pT7-3/p1ΔC11	pT7-3/p1ΔC11 → BL21(DE3) (Amp)
B. subtilis		
168	*trpC2,* considered WT strain	*Bacillus* Genetic Stock Center
SWV215	*trpC2 pheA1 Ω(spo0A::kan)*	Xu and Strauch (1)
168Δ*spo0A*	*trpC2 Ω(spo0A::kan)*	SWV215 → 168 (K_m)
168/YFP-p1	*trpC2 Ω(amyE::*P_{xyl}*-yfp-p1 spc)*	pSG5472/YFP-p1 → 168 (Sp)
DBP-001	*trpC2 Ω(amyE::*P_{xyl}*-yfp-p1 spc) Ω(spo0A::kan)*	SWV215 → 168/YFP-p1
1057	*trpC2 Ω(amyE::*P_{xyl}*-ftsZ–cfp spc)*	Feucht and Lewis (2)
DBP-002	*trpC2 Ω(amyE::*P_{xyl}*-yfp-p1 spc) Ω(thrC::*$P_{hyper\text{-}spank}$*-ftsZ-cfp erm)*	pSG5472/YFP-p1, pDP150/FtsZ-CFP → 168 (Sp, Erm)
1801	*trpC2 chr::pJSIZΔpble (*P_{spac}*–ftsZ ble)*	Marston et al. (3)
DBP-003	*trpC2 chr::pJSIZΔpble (*P_{spac}*–ftsZ ble) Ω(amyE::*P_{xyl}*-yfp-p1 spc)*	pSG5472/YFP-p1 → 1801 (Sp)
804	*trpC2 Ω(ftsL::pSG441 aphA-3* P_{spac}*-pbpB neo)799*(ϕ105J506) *cat* P_{xyl}*-ftsL*	Daniel et al. (4)
DBP-004	*trpC2 Ω(ftsL::pSG441 aphA-3* P_{spac}*-pbpB neo)799*(ϕ105J506) *cat* P_{xyl}*-ftsL Ω(amyE::*P_{xyl}*-yfp-p1 spc)*	pSG5472/YFP-p1 → 804 (Sp)
168ftsZHis	*trpC2 Ω(ftsZ::pMUTinHisΔftsZ)*	Ishikawa et al. (5)
168His	*trpC2 Ω(ftsZ::pMUTinHisΔftsZ) Ω(spo0A::kan)*	SWV215 → 168ftsZHis (K_m)
DBP-005	*trpC2 Ω(thrC::*$P_{hyper\text{-}spank}$*-p1 erm)*	pDP150/p1 → 168 (Em)
DM-024	*trpC2 Ω(thrC::*$P_{hyper\text{-}spank}$*-cfp erm)*	Muñoz-Espín et al. (6)
JH642	*trpC2 pheA1*	Perego et al. (7)
PL1780	JH642 *ezrA*::*ezrA*(*R510D*) *spc*	Haeusser et al. (8)
DBP-006	*trpC2 pheA1 Ω(spo0A::kan)*	SWV215 → JH642 (K_m)
DBP-007	JH642 *ezrA*::*ezrA*(*R510D*) *spc Ω(spo0A::kan)*	SWV215 → PL1780 (K_m)
110NA	*trpC2 spo0A*$^-$ *su*$^-$	Moreno et al. (9)

*Antibiotic resistance gene abbreviations are as follows: *ble*, bleomycin; *erm*, erythromycin; *kan*, kanamycin; *neo*, neomycin; *spc*, spectinomycin.
†X → Y indicates that strain Y was transformed with DNA from source X, with selected marker in parentheses. Amp, ampicillin; Em, erythromycin; K_m, kanamycin; Sp, spectinomycin.

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Table S6. ϕ29 phages used

Phage	Source or reference
ϕ29 WT	Laboratory stock
ϕ29 *sus14*(1242)	Jiménez et al. (1)
ϕ29 *sus1*(629)	Reilly et al. (2)

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Table S7. Plasmids used

Plasmids	Relevant features	Reference
pSG5472	pSG1729 derivative containing *yfpmut2* gene instead of *gfpmut1*	Muñoz-Espín et al. (1)
pSG5472/YFP-p1	pSG5472 containing *yfp*::p1 fusion	This work
pDP150	pDR111 derivative *thrC* integrating vector containing $P_{hyper\text{-}spank}$	Kearns and Losick (2)
pDP150/FtsZ-CFP	pDP150 containing *ftsZ*::*cfp* fusion	This work
pDP150/p1	pDP150 containing ϕ29 *gene 1*	This work
pT7-3	a T7 RNA polymerase promoter based vector	Tabor and Richardson (3)
pT7-3/p1ΔC11	pT7-3 containing ϕ29 *gene 1ΔC11*	This work

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